

iGEM 2016: Team Pittsburgh

Week 21 Lab Notebook

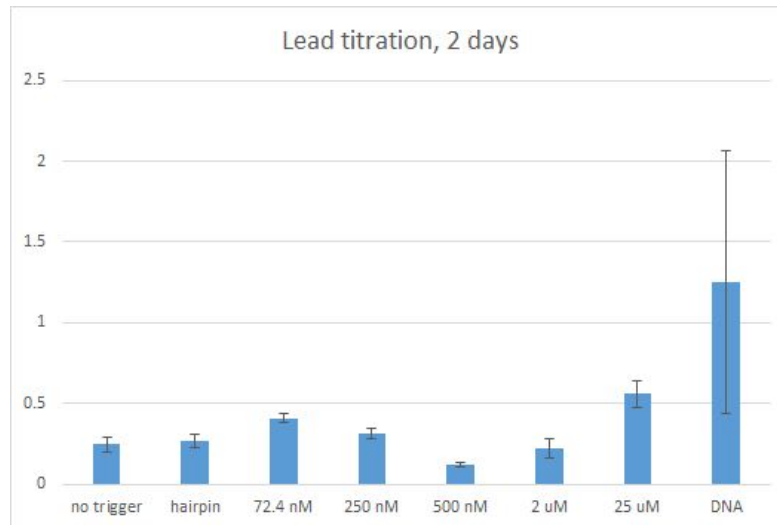
Monday, October 10

Liquid cultures of lacZ mutagenesis (Maya)

Linearize RBS-T3 with XbaI and EcoRI (Maya)

Anneal PT3 (Maya)

Saturday's cell-free reactions darkened and evaporated-- +10 μ L H₂O and read (Claire)



Tuesday, October 11

Re-streak cells (Claire)

CMU codon-optimized proteins, CM

G switch, kanamycin

Competent cells, LB

Wednesday, October 12

Liquid cultures (Claire)

Codon-optimized proteins: 4 each (dense growth, difficult to pick individuals)

Competent cells: 4

G switch: 8

50 mg/mL kanamycin sulfate

Thursday, October 13

LacZ Mutagenesis with Q5

Linearize PT7-Toehold with EcoRI and PstI

Scan overnight cultures of proteins (Claire with Cheryl)

Time course of proteins, 1 of each overnight culture diluted 1:50

Miniprep G switch (Claire)

Friday, October 14:

Ligate

PT3 to RBS-T3

Cell-free lead titration and mismatch test

A1-2	no trigger	1.2 μ L H ₂ O
3-4	hairpin only, 3.74 nM	1 μ L hp @ 74.8 nM 1 μ L H ₂ O] 1.2 μ L hp @ 37.4 nM
	3.74 nM hp + 50 nM lead	1 μL hp @ 74.8 nM 1 μL Pb²⁺ @ 1 μM] 1.2 μL
6	3.74 nM hp + 72.4 nM lead	1 μ L hp @ 74.8 nM 5 μ L Pb ²⁺ @ 1.448 μ M] 1.2 μ L
	+ 100 nM Pb²⁺	1 μL hp @ 74.8 nM 1 μL Pb²⁺ @ 2 μM] 1.2 μL
7-8	+ 250 nM Pb²⁺	1 μL hp @ 74.8 nM 1 μL Pb²⁺ @ 5 μM] 1.2 μL
9-10	+ 500 nM Pb ²⁺	1 μ L hp @ 74.8 nM 1 μ L Pb ²⁺ @ 10 μ M] 1.2 μ L
11-12	+ 2 μ M Pb ²⁺	3 μ L hp @ 74.8 nM 3 μ L Pb @ 40 μ M] 1.2 μ L
13-14	+ 25 μ M Pb ²⁺	1 μ L hp @ 74.8 nM 1 μ L Pb @ 500 μ M] 1.2 μ L
	+ 50 μM Pb²⁺	1 μL hp @ 74.8 nM 1 μL Pb @ 1 nM] 1.2 μL
15-16	3.74 nM DNA	1.2 μ L DNA @ 37.4 nM

Switch Mismatch Cell-free test

G, no trigger

D, no trigger

G, hp only

D, hp only

G, 3.74 nM hp + 2 μ M lead

D, 3.74 nM hp + 2 μ M lead

G, DNA

D, DNA

16 rxns, 1

Sol'n A: 2.4

52.8

B 1.8

39.6

RNAse 0.3 x 22 = 6.6

substrate 0.3 6.6

105.6

$\frac{105.6}{2} = 52.8 \mu$ L per switch.

G: 52.8 μ L master

D: 52.8 μ L master

13.2 μ L switch

13.2 μ L switch @

@ 50 ng/ μ L

50 ng/ μ L

no trigger

1.2 μ L H₂O

62

hp only

1.2 μ L 37.4 nM hp

hp + lead

1.2 μ L hp + Pb

G DNA trig

1.2 μ L @ 37.4 nM

Friday, October 14.

Cell-free lead titration & mismatch (pp110-112)

28 rxns (14 conditions)

Sol'n A	2.4	76.8
B	1.8	57.6
RNAse	0.3	9.6
substrate	0.3	9.6
		38.4
		153.6 - 115.2 → D

6.1 G D (38.4) cond.
MM = ~~13.4~~ 115.2 (12 cond), Master mix 17.2 μ L (4 rxns)
50 μ L switch = 14.4 μ L 59.7 μ L switch 76.8 μ L (25 ng)
63.1 μ L switch = 9.51 μ L H₂O 4.85 μ L 50 μ L switch
H₂O = 4.89 μ L 25 μ L 59.7 μ L + 4.85 μ L H₂O = 50 μ L
10.8 μ L / rxn 10.8 μ L / rxn
- no trigger (1.2 μ L H₂O)
- 3.74 nM hp
- 3.74 nM hp + 2 μ M lead.

Forgot 72.4 nM condition

put tubes in incubator for ~2 min, removed to
add DNA to DNA trig. cond, replace in 37. 2 hr. 40 min.

G no trigger	A 17-18
hairpin	A 19-20
50 nM Pb	A 21-22
100 nM	A 23-24
250 nM	B 1-2
500 nM	B 3-4
2 μ M	B 5-6
25 μ M	B 7-8
50 μ M	B 9-10
DNA	B (11-12) low volume
D no	B 13-14
hairpin	B 15-16
2 μ M Pb	B 17-18

